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RAPID REPORT

SLEEP AND REST FACILITATE AUDITORY LEARNING

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Abstract—Sleep is superior to waking for promoting performance improvements between sessions of visual perceptual (Stickgold et al., 2001) and motor (Smith and MacNeill, 1994; Fischer et al., 2002; Walker et al., 2002) learning tasks. Few studies have investigated possible effects of sleep on auditory learning (Fenn et al., 2003; Atienza et al., 2004). A key issue is whether sleep specifically promotes learning, or whether restful waking yields similar benefits (Tononi and Cirelli, 2001). According to the "interference hypothesis," sleep facilitates learning because it prevents interference from ongoing sensory input, learning (e.g., Walker et al., 2003a) and other cognitive activities that normally occur during waking. We tested this hypothesis by comparing effects of sleep, busy waking (watching a film) and restful waking (lying in the dark) on auditory tone sequence learning. Consistent with recent findings for human language learning (Fenn et al., 2003), we found that compared with busy waking, sleep between sessions of auditory tone sequence learning enhanced performance improvements. Restful waking provided similar benefits, as predicted based on the interference hypothesis. These findings indicate that physiological, behavioral and environmental conditions that accompany restful waking are sufficient to facilitate learning and may contribute to the facilitation of learning that occurs during sleep.

Key words: sleep, napping, rest, auditory perceptual learning, retroactive interference, plasticity.

Sleep may promote learning and neural plasticity (Buzsáki, 1989; Sejnowski and Destexhe, 2000; Tononi and Cirelli, 2001). Sleep stages (Horne and Walmsley, 1976; Cantero et al., 2002), as well as electroencephalogram (EEG) slow-wave activity (Kattler et al., 1994; Vyazovskiy et al., 2000; Miyamoto et al., 2003), regional cerebral blood flow (e.g. Peigneux et al., 2003) and neuronal activity (e.g. Wilson and McNaughton, 1994; Nádasdy et al., 1999; Dave and Margoliash, 2000; Hirase et al., 2001) during sleep are related to the activity of the brain during prior wakefulness. These changes in sleep as a result of waking activity may reflect processes involved in synaptic reorganization (Buzsáki, 1989, 1998; Tononi and Cirelli, 2003). Consistent with a functional role of sleep in neural plasticity, sleep enhanced visual cortical plasticity induced by monocular deprivation during a critical period of development in cats (Frank et al., 2001). Numerous human and animal studies suggested that rapid eye movement (REM) or non-REM sleep or both improve memory retrieval (reviewed in Sejnowski and Destexhe, 2000; Stickgold et al., 2001; Tononi and Cirelli, 2001). Increasing evidence suggests that sleep promotes visual perceptual (reviewed in Stickgold et al., 2001) and motor (Smith and MacNeill, 1994; Fischer et al., 2002; Walker et al., 2002) learning in humans. However, the proposed involvement of sleep in learning and memory remains controversial (e.g. Vertes and Eastman, 2000; Siegel, 2001).

The present study addressed the question of whether sleep specifically facilitates learning, or whether resting can provide similar benefits (Tononi and Cirelli, 2001). We investigated the effect of an afternoon nap on learning of a challenging auditory tone perception task. The nap paradigm had the advantage that it permitted waking control groups without necessitating sleep deprivation. Napping was previously found to have large effects on visual perceptual learning (Mednick et al., 2003).

EXPERIMENTAL PROCEDURES

Subjects

Participants ($n=64$) were 18–28 year-olds with hearing thresholds ≤35 dB hearing level (250–3000 Hz). They were right-handed nonsmokers with moderate alcohol (≤5 drinks/week) and caffeine (≤3 cups coffee/day) consumption, no flights involving time shifts for 2 months before the study, and no history of neurologic or psychiatric disease. For 3 days before the study subjects maintained a regular sleep schedule (23:00–07:00 h, verified by wrist actimetry), refrained from napping, and abstained from alcohol and caffeine. Procedures were approved by the local ethics committee and subjects gave written informed consent.

Design

Subjects were randomly assigned to sleep, restful waking (RW), busy waking (BW) or no-break (NB) groups ($n=16$ per group), with the constraints that groups were matched in age and gender. In each group, mean age was 23 years and half of the subjects were female. The experimental period started at 13:55 or 15:55 h (half of each group at each time). Experiment times and gender were completely counterbalanced. In the NB group, subjects completed immediately consecutive learning sessions that began or ended at times matched to those of other groups (one quarter of the sub-

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Abbreviations: BW, busy waking; EEG, electroencephalogram; NB, no break; rANOVA, repeated measures analysis of variance; REM, rapid eye movement; RT, reaction time; RW, restful waking.

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Table 1. Timing of experimental procedures

Procedure	Time[1]
Session 1: Auditory learning task, preceded and followed by a subjective sleepiness rating[2]	12:45–13:30
Restroom break, snack, impedance tests, subjects informed of group assignments	13:30–13:55
Experimental manipulation, concurrent polysomnographic recording	13:55–15:55
Sleep group: sleep opportunity period	
RW group: lay awake in bed in dark room	
BW group: watched film	
Time to permit recovery from sleep inertia with reaction time and addition tests, subjective sleepiness ratings, and rest breaks supervised in the laboratory	15:55–17:00
Session 2: Auditory learning task, preceded and followed by a subjective sleepiness rating[2]	17:00–17:45

[1] Half of the subjects in the sleep, RW, and BW completed the procedures at the times given and half completed the procedures 2 h later. In the NB group, subjects completed immediately consecutive learning sessions, such that session 1 or session 2 was performed at times matched to those of other groups (one quarter of the subjects at each time).

[2] A German translation of the Stanford Sleepiness Scale was used.

jects at each time). The level achieved, learning score, and reaction times did not differ among groups in session 1, indicating no differences in their ability to perform the task.

Procedure

Procedures are summarized in Table 1. Electrodes were applied to sleep, rest, and wake groups for polysomnographic recordings during the experimental period. Subjects completed two 45-min sessions of the auditory learning task (Fig. 1a). After the first session, subjects had a 25-min break when electrode impedances were checked and subjects were informed of group assignments. During the immediately following 2-h experimental period, subjects in sleep and RW groups lay in bed in a darkened single bedroom. The RW group was instructed to relax without falling asleep. If rolling eye movements or reduced alpha activity appeared, we alerted subjects by sounding a tone over the intercom. BW subjects sat in a lighted room under experimenter supervision and watched an educational film. They were instructed to attend carefully because they would answer questions about the film afterward. To allow time for recovery from sleep inertia, the second learning session began 65 min after the experimental period. Subjects spent intervening time in the same controlled manner (with short tests and rest breaks) in the laboratory. The NB group completed the two learning sessions with no intervening time between sessions.

Data analysis

The alpha criterion for statistical analyses was 0.05. The level achieved was analyzed with ordinal logistic regression. The learning score assessed performance accuracy and weighted correct answers for each level based on theoretical considerations of difficulty (Fig. 1b). Zero points were given for incorrect answers. The number of points a subject receives for a correct answer should be inversely related to the probability of getting a correct answer by chance, because an improbable correct answer is more likely to reflect knowledge. In level 1, the probability of getting a correct answer by chance is 1/4, so four points were given for a correct answer. Upon reaching level 2, subjects had already learned to discriminate single patterns. Thus, it is assumed that one of the two patterns (e.g. the square) is identified correctly, leading to a 1/3 probability of getting the second pattern correct by chance. Seven points are given for correctly identifying the sequence, because the first pattern is worth four points (as in level 1), and identifying the second pattern is worth three points (reciprocal of the chance probability). Following analogous considerations, correct answers received nine points in level three and 10 points in level 4. Learning scores were averaged over all trials in each session and subjected to repeated measures analysis of variance (rANOVA) with group and session factors. Paired *t*-tests (two-tailed) assessed changes in performance between sessions. We used one-tailed unpaired *t*-tests to test predictions that performance changes in (1) the sleep and the RW groups would be greater than the BW group; (2) the sleep group would be greater than the RW group; (3) the NB group would be greater than the BW group; (4) the sleep and RW groups would be greater than the NB group. Predictions were based on the interference hypothesis and on previous findings (Mednick et al., 2002; Walker et al., 2002; Fenn et al., 2003).

Reaction times (RT; latency from stimulus onset to final button press) from the first block of level one (approximately the first 5 min of the task) were transformed (1/RT), analyzed with rANOVA and reverse transformed (Fig. 2b).

RESULTS AND DISCUSSION

The sleep group slept for 77 min on average (Table 2), and all individuals slept for more than 30 min. Two subjects in the RW group and one subject in the BW group exhibited very short periods of sleep that constituted 0.3–4% of the experimental period. Excluding these subjects did not alter statistical results. Previous studies of human sleep and learning have generally not included polysomnographic recordings of waking groups. Awareness of recordings motivates participants and their supervisors to ensure that wakefulness is maintained; the occurrence of microsleep despite this awareness underscores the advantages of conducting polysomnographic recordings in waking groups.

On the auditory learning task, the maximum level achieved increased from session 1 to session 2 ($\chi^2=3.83$, $P=0.05$), indicating overall improvement. The change in level achieved did not differ among groups ($\chi^2=2.25$, $P=0.52$). Three, 57, and four subjects respectively reached levels 1, 2, and 3 in the first session; 55 subjects, eight subjects, and one subject respectively reached levels 2, 3, and 4 in the second session.

The learning score (Fig. 1b) provided a more sensitive measure of performance accuracy. Changes in learning scores from session 1 to session 2 differed among groups (Fig. 2a), as indicated by a significant group×session interaction ($F(3,60)=3.59$, $P=0.02$). Sleep and RW groups showed significant improvement from session 1 to session 2; the BW group did not. Although performance improve-

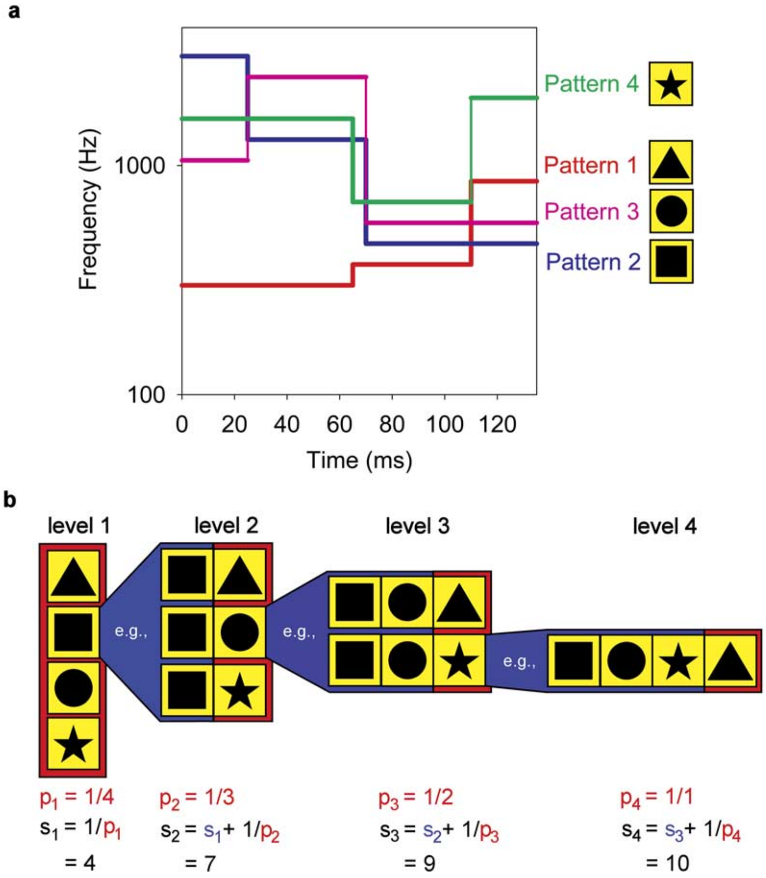

Fig. 1. Auditory learning task (based on Leek and Watson, 1988). (a) Auditory tone patterns. Subjects identified pattern(s) by pressing corresponding button(s) labeled with the symbols shown. Stimuli were presented in blocks of 48 trials at 70 dB sound pressure level. Initially, single patterns were presented. Visual feedback indicated whether the response was correct or incorrect and the correct answer was shown. After each block, percentage correct for that block was displayed. If subjects obtained ≥90% correct, the length of the stimulus sequence was increased by one pattern in the next block. For example, if a subject obtained ≥90% correct in identifying single patterns (level 1), subsequently sequences of two patterns were presented (level 2). Sequence length could increase up to four patterns. The interval between patterns within a sequence was 50 ms. The same pattern never occurred more than once within a sequence. Accuracy was emphasized over speed. (b) Learning scores. Performance in each level was weighted according to theoretical considerations of difficulty (see Experimental Procedures). The actual accuracies achieved support our weighting system. In the first block of levels 1 and 2 in the first session, subjects achieved means of 80±1% and 43±2% correct. The theoretical weighting of 1.8 closely resembles the relative empirical difficulty of 1.9. p_i, probability of correct answer in level i; s_i, score for correct answer in level i.

ment in the sleep group was greater than in the RW group, this difference did not reach statistical significance. Performance increases in both sleep and RW groups were significantly larger than in the BW group.

As predicted based on the interference hypothesis, the sleep and RW groups showed greater improvement than did the BW group. Whereas sensory input was dramatically reduced in sleep and RW groups, the BW group watched a film involving visual and auditory stimulation. Interference due to ongoing stimulation may have prevented performance improvements in the BW group. Previous studies provided only partial control for such interference (Mednick et al., 2002; Walker et al., 2002). To prevent waking activities from interfering with learning, it may be necessary to limit motor activity and sensory input from all modalities, as in the RW group of the present study.

Another possible source of waking interference is learning. Learning while watching the film may have interfered with the BW group's performance in the second auditory learning session. Learning new information can interfere with recall of previously learned information, a phenomenon known as retroactive interference. Based on the finding that subjects re-learned a list of syllables more quickly if they memorized it immediately before going to bed than if they memorized it in the daytime (Heine, 1914), Heine suggested that sleep benefits learning by preventing retroactive interference that normally occurs during waking. Our results are consistent with Heine's interpretation.

Physiological processes common to sleep and RW may actively facilitate learning and serve to prevent waking interference. Neuronal replay that is postulated to facilitate learning (e.g. Wilson and McNaughton, 1994; Nádasdy et al., 1999; Dave and Margoliash, 2000) could occur during RW as well as during sleep. Hippocampal sharp waves may provide a mechanism for neuronal replay (Nádasdy et al., 1999) and consequent neural plasticity, and they occur

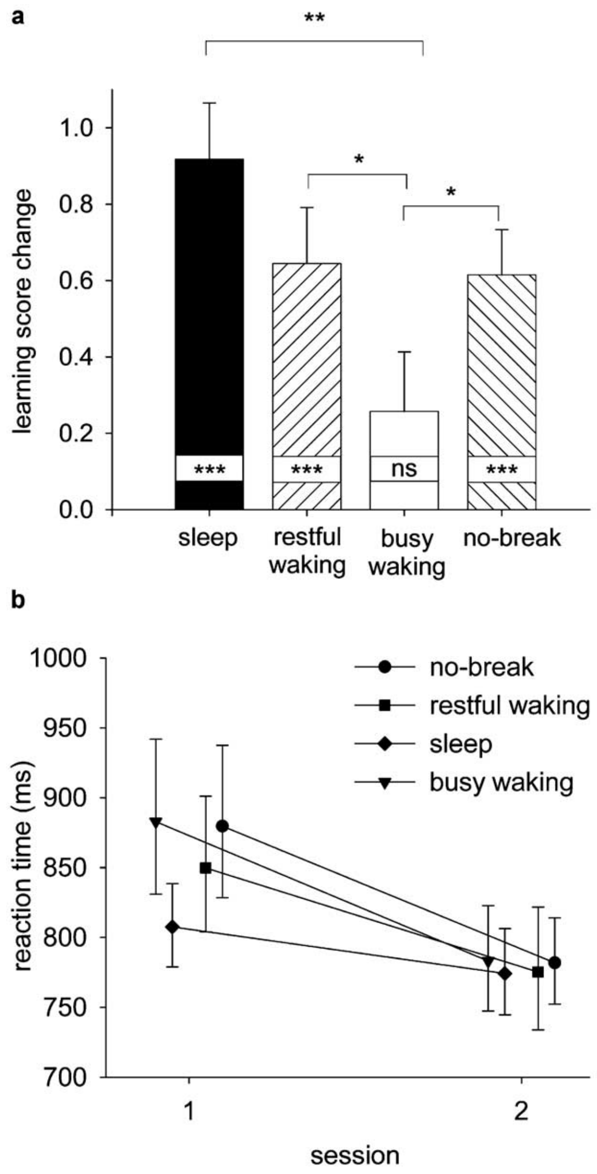

Fig. 2. Changes in performance from session 1 to session 2. (a) Changes in learning scores. Increases of 24%, 18%, 6%, and 18% were observed in sleep (s), RW, BW, and NB groups, respectively. Performance in session 1 did not differ among groups ($F(3,60)=0.90$, $P=0.45$). Symbols on bars indicate changes between sessions. P-value symbols: $***$ <0.001, $**$ <0.01, $*$ <0.05; ns, not significant. Statistics for differences between sessions: $t_s=6.22$, $P_s<0.0001$, $t_{rw}=4.47$, $P_{rw}=0.0004$, $t_{bw}=1.68$, $P_{bw}=0.11$, $t_{nb}=5.18$, $P_{nb}=0.0001$. Statistics for differences between groups: $t_{s\text{-}bw}=3.07$, $P_{s\text{-}bw}=0.002$; $t_{rw\text{-}bw}=1.82$, $P_{rw\text{-}bw}=0.04$; $t_{nb\text{-}bw}=1.81$, $P_{nb\text{-}bw}=0.04$; $t_{s\text{-}nb}=1.60$, $P_{s\text{-}nb}=0.06$; $t_{s\text{-}rw}=1.31$, $P_{s\text{-}rw}=0.10$; $t_{rw\text{-}nb}=0.18$, $P_{rw\text{-}nb}=0.43$. (b) Reaction times. Performance became faster from session 1 to session 2 ($F(1,60)=15.47$, $P<0.001$). This change did not differ among groups (group×session interaction: $F(3,60)=0.54$, $P=0.66$). Speed of performance did not differ among groups in session 1 ($F(3,60)=0.58$, $P=0.63$). Error bars indicate S.E.M.

during immobile waking as well as during slow wave sleep (Buzsáki, 1989, 1998).

Table 2. Sleep variables

Variable	Duration (min)[1]
Sleep latency[2]	13.6±2.1
Stage 1	6.1±0.8
Stage 2	39.4±4.2
Stage 3	9.2±1.9
Stage 4	19.2±4.2
Slow wave sleep (stages 3 and 4)	28.3±4.2
REM sleep[3]	13.6±2.2
REM sleep latency[3]	64.0±4.0
Total sleep time	77.1±5.6
Waking after sleep onset	16.3±4.1
Sleep efficiency[4] (%)	64.4±4.7

[1] Data from the sleep group, $n=16$.

[2] Latency to stage 2.

[3] Eleven of the subjects exhibited REM sleep, and only these $n=11$ were included in calculating REM sleep duration and latency.

[4] The percentage of time when the subjects were asleep (stages 2, 3, 4, or REM sleep) relative to the total time in bed.

If sleep and rest actively facilitate learning, one might expect greater between-session improvement if subjects sleep or rest between sessions than if they complete immediately consecutive sessions. However, improvement in the sleep and RW groups did not differ significantly from the NB group. This finding does not preclude the possibility that sleep and rest actively promote learning, because continuous learning processes and between-session performance improvements might involve different mechanisms (Walker et al., 2003b). The performance increase in the NB group was significant and larger than in the BW group (Fig. 2a), as expected based on the interference hypothesis. A previous study likewise showed that a group with no break between language learning sessions showed greater between-session improvement than a group that was awake for 12 h between sessions, while the NB group did not differ from a group that had a night of sleep (Fenn et al., 2003).

Improved attention after sleep or rest might reduce retroactive interference or contribute to subsequent performance improvements by enhancing automaticity of brain responses to auditory stimuli (Atienza et al., 2004). Potentially decreased concentration due to sustained attention might have contributed to the lack of improvement in the BW group, yet the NB group sustained attention to the auditory learning task for the longest continuous period and nonetheless showed larger improvements than did the wake group. Moreover, BW, RW and sleep groups rated their subjective sleepiness similarly (Table 3), and reaction times on the learning task did not differ among groups (Fig. 2b), suggesting that differences in alertness (e.g. residual sleep inertia) cannot account for the results.

Compared with BW, sleep facilitated learning of auditory tone patterns. Subjects who rested or performed continuously also showed performance improvements, and these improvements did not differ significantly from those observed in the sleep group. These findings support the idea that sleep and rest promote auditory learning by reducing interference that normally occurs during waking. Other physiological, behavioral and environmental changes common to sleep and rest may also facilitate

Table 3. Subjective sleepiness ratings

	Before session 1	After session 1	Before session 2	After session 2
BW	2.44±0.20	3.00±0.30	2.81±0.28	3.19±0.29
RW	2.19±0.16	3.31±0.22	2.63±0.26	3.13±0.27
Sleep	2.13±0.20	3.06±0.28	2.38±0.20	2.69±0.25

[1] Values are means±standard errors ($n=16$ per group). Ratings on a scale of 1 to 7 were made using the Stanford Sleepiness Scale; higher ratings correspond to greater sleepiness. rANOVA showed no differences between groups (group main effect: $F(2,45)=3.26$, $P=0.45$, group×time interaction: $F(6,135)=0.63$, $P=0.68$, Huynh-Feldt $\varepsilon=0.86$). NB subjects did not rate their sleepiness.

learning. Our findings do not exclude the possibility that sleep-specific processes provide additional benefits to auditory learning beyond the benefits of RW; the sleep group showed greater improvement than the RW group, although this difference did not reach significance. Comparing sleep with resting conditions and quiescent states such as meditation or hypnosis promises to enrich future investigations of the influence of sleep on learning.

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